Brief Rapid Communication

Intravascular Ultrasound Imaging of Coronary Arteries

Is Three Layers the Norm?

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Background. **The purpose of this study was to evaluate the significance of the three-layered appearance of coronary arteries in adolescence and adults from intravascular ultrasound scans and to correlate these observations with histopathology.**

Methods and Results. **Sixteen intact hearts were excised at autopsy from patients with no clinical history of coronary artery disease. The patients' ages ranged from 13 to 55 years. A 30-MHz ultrasound imaging catheter was used to obtain images throughout the epicardial coronary vasculature. A total of 72 image cross sections was marked by epivascular sutures, and the corresponding histological sections were examined. Ultrasound images were classified into two groups: images exhibiting three-layered appearance and images without distinct layering. Histological analysis revealed a significantly greater degree of intimal thickening in segments with three layers (243±105 μm) than in nonlayered segments (112±55 μm). Discriminant analysis of these data predicted the threshold between the two groups to be 178 μm. Measurements of medial thickness were not different between these two groups (235±61 versus 210±76 μm). In the nonlayered group, the average patient age was 27.1±8.5 years, whereas in the three-layered groups, the average age was 42.8±9.8 years.**

Conclusions. **The intracoronary ultrasound image appearance of young, morphologically normal coronary artery walls is homogeneous without layering. A three-layered appearance suggests the presence of at least 178 μm of intimal thickening and is seen more frequently with advancing age. (*Circulation* 1992;86:154–158)**

KEY WORDS • **intravascular ultrasound imaging** • **coronary arteries** • **intimal thickness**

High-frequency intravascular ultrasound is a promising new technology that is able to demonstrate the boundaries of vessel wall layers in vivo.[1–5] The ultrasound backscatter from the vessel wall is proportional to the acoustic impedance difference between layers and the spatial distribution of the inhomogeneities within each layer. Early studies with intravascular ultrasound in peripheral vessels demonstrated a characteristic three-layered ultrasound appearance in normal, nondiseased arteries.[6,7] These studies showed relatively bright intimal and adventitial signals separated by a hypoechoic region, corresponding histologically to the medial layer. This three-layered appearance became largely accepted as the characteristic appearance of normal muscular arteries.

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High-quality coronary artery images have become available over the past year, and several investigators have observed that the three-layered appearance in these vessels is variable.[8–10] Even with the excellent resolution afforded by 30-MHz images, we have noted that the three-layered appearance may be absent, particularly in the vessels from younger patients. On this basis, we hypothesized that a certain degree of intimal thickening may be necessary in order for the three-layered appearance to be manifested, i.e., that the three layers may not in fact represent the pattern from a truly normal coronary segment.

To test this hypothesis, we examined a series of coronary artery segments from freshly explanted human hearts and compared the histological results with the ultrasound appearance of layering. Because the backscatter is related to vessel orientation, the pressure within the vessel plays a key role in the ultrasonic appearance and hence, layering of vessel walls.[11] Thus, in this study, tissue specimens were histologically prepared at pressure identical to that during the corresponding image acquisition. In a second phase of the study, we performed clinical imaging studies with freshly transplanted hearts from young donors in which a minimal degree of intimal thickening would be expected.

Methods

Imaging System

All in vitro and in vivo studies were performed using a commercially available intravascular ultrasound system (CVIS, Sunnyvale, Calif.). Images were obtained with a 5F, 30-MHz ultrasound catheter. This catheter-based system provides real-time images of vessel cross sections with an axial resolution (−3-dB impulse response width) of 75 μm and lateral resolution (1.1-mm-diameter circular transducer) of approximately 150 μm. Actual resolution on the video monitor may be as much as 50% less, depending on the image dynamic range. The ultrasound system used for this study generates a video signal with a dynamic range of approximately 55 dB. During each study, the gain, reject, and compression settings were adjusted to generate images with the largest gray-scale range. All vessel images were located within the focal zone of the transducer, which was between 1.5 and 4.5 mm. Images were recorded on high-quality videotape (sVHS, 0.5 in.) for off-line review.

In Vitro Image Acquisition

Sixteen intact hearts were excised at autopsy from 12 male and four female patients with no clinical history of coronary artery disease. The patients' ages ranged from 13 to 55 years. The excised hearts were studied within 6 hours of harvesting. An 8F guiding catheter was inserted into the left coronary sinus and seated deeply in the left main coronary artery. The left main artery was isolated from perivascular tissue, and a ring suture was placed around the vessel to form a tight seal between the vessel and guiding catheter. Constant flow with saline was established at a pressure of 100 mm Hg throughout the left coronary vessels.

The imaging catheter was inserted into the coronary vasculature over a 0.014-in. standard angioplasty guide wire. Multiple high-quality images were selected in the left anterior descending and circumflex distributions for each heart, and epicardial sutures were placed in these locations for histological comparison.

In Vivo Image Acquisition

Four male cardiac transplant patients (donor ages, 19, 23, 29, and 34 years) were imaged with the 5F, 30-MHz catheter during routine predischarge catheterization. Angiograms in these cases showed no evidence of coronary artery disease. For each study, the left anterior descending coronary artery was imaged along its entire length to the apex. Two of these patients died of noncardiac causes within 3 months of transplantation. The hearts from these patients were excised and studied in vitro according to the same methods as in the in vitro acquisition. These two patients provided the unique opportunity to compare the observations found during in vivo intracoronary imaging with pressure-fixed histology at autopsy.

Image Classification

All coronary images from both in vitro and in vivo studies were classified into two groups: 1) the clear appearance of three layers for >75% of the lumen circumference (three-layered group) and 2) no appearance of three layers for >75% of the lumen circumference (nonlayered group).

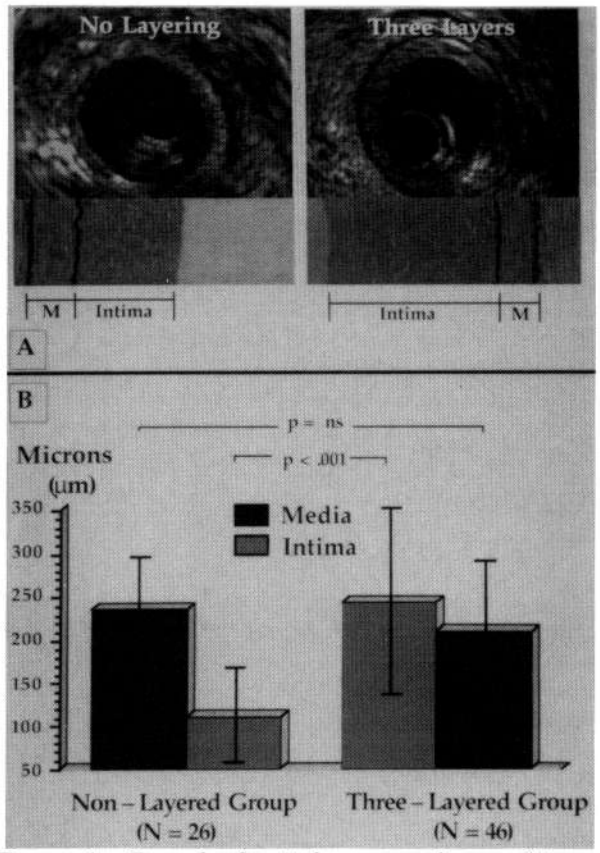

FIGURE 1. *Example of typical coronary ultrasound images with and without three-layered appearance* (panel A). Panel B: *Graph demonstrates the distribution of mean intimal thickness and mean medial thickness of the nonlayered group and the three-layered group. Data are expressed as mean ±1 SD. M, media.*

We chose to evaluate layering in the entire circumference of a given cross section rather than on a point-by-point basis for several reasons. Small alterations in vessel size and shape during fixation makes precise comparisons between the ultrasound images at a particular point and the corresponding histological radius difficult. In addition, the appearance of layering on the ultrasound images is significantly influenced by the distance between the transducer and the vessel wall and the angle between the plane of the beam and the wall. By analyzing the entire circumference of the vessel, these effects are potentially minimized through averaging.

Figure 1A shows two in vitro coronary images that were pressure-perfused at 100 mm Hg, demonstrating clear layering (right) and the lack of circumferential three-layered appearance (left). Of the 72 image cross sections classified, 46 were classified in the three-layered group (63%) and 26 were classified in the nonlayered group (37%).

Histological Measurements

After image acquisition of the perfused coronary arteries, the perfusate of saline was changed to 10% neutral buffered formalin, maintaining a constant pressure of 100 mm Hg, and allowed to fix for a minimum of 6 hours. Transverse arterial sections were obtained at

the level of the epicardial sutures. These vessel cross sections were prepared with both hematoxylin and eosin and elastin–van Gieson's (EVG) stains.

Pressure-fixed, EVG-stained specimens were microscopically examined (×100), and the intimal thickness was measured from the luminal border to the internal elastic lamina. Around the lumen circumference, an intimal thickness measurement was made at 45° intervals, and a total of eight measurements were averaged to obtain the mean intimal thickness (MIT) for each vessel cross section. Medial thickness was defined as the distance between the inner adventitial border and the internal elastic lamina. Mean medial thickness (MMT) was obtained by averaging equally spaced measurements of media around the circumference of the vessel. All histological dimensions are reported in micrometers.

Statistical Methods

A total of 72 image cross sections were compared with the corresponding 72 histological samples. Each MIT and MMT from the histological sections was separated according to the two image groups and expressed as mean±SD. Discriminant analysis of MIT was performed using SAS/STAT (DISCRIM) software (SAS Institute, Inc., Cary, N.C.). Classification was done using a parametric model, assuming that each group (three-layered and nonlayered) had a multivariate normal distribution. With prior probabilities set to equality, the analysis generated a linear discriminant function or threshold criteria between the two groups. This threshold value of MIT is reported in micrometers.

Results

In Vitro Results

The distribution of the MITs and MMTs for the three-layered group and the nonlayered group is shown in Figure 1B. The average MIT for the three-layered group is 243±105 μm. This value is significantly larger than the average MIT for the nonlayered group (112±55 μm). Discriminant analysis between these two distributions results in a threshold level of 178 μm. On the other hand, the average MMT for the three-layered group (210±76 μm) was not significantly different from the nonlayered group (235±61 μm). Thus, from these in vitro data with an ultrasound frequency of 30 MHz, the emergence of the typical three-layered appearance of coronary arteries occurs when the intimal thickness exceeds 178 μm. In other words, for this particular scanner, the presence of three layers in intravascular ultrasound imaging of coronary arteries indicates mild disease.

The average age of patients in the three-layered group was 42.8±9.8 years. This is in contrast to the nonlayered group, in which the average age was 27.1±8.5 years. Figure 2 illustrates coronary ultrasound images from four patients of different ages. In the older patients (panels A and C), the intimal thickness exceeds 178 μm, and the cross-sectional scans exhibit three layers. Conversely, the younger patients (panels B and D) demonstrate no vessel wall layering. Thus, coronary arteries with minimal or no disease exhibit a uniform vessel wall appearance.

In Vivo Results

Using the criteria for three layers as seen with intracoronary ultrasound, we found that no patient exhibited layering in either the left anterior descending or circumflex distributions. All angiographic studies were completely normal. Figures 2B and 2D display representative images of the transplant patients imaged. Note that there is no appearance of layering in these vessel cross sections.

In the transplant hearts imaged postmortem, the in vitro image findings were similar to those observed when these same vessels were studied in vivo. That is, there was no layering observed within the vessel wall on the ultrasound image. Histological specimens from both cases revealed an average MIT that was less than the 178 μm. Thus, the in vitro results would predict nonlayering for the in vivo intravascular images. Figure 2B is an image from one of these transplant patients demonstrating the lack of circumferential vessel wall layering. The pressure-fixed histological sample from the proximal left anterior descending coronary artery of this patient showed the intimal layer to have an average thickness of 134±31 μm. This value for the MIT is near the threshold of thickness needed to generate ultrasound layering at 30 MHz. In this image, there is a suggestion of layering in the 12–2 o'clock region that may indicate that the absolute intimal thickness exceeds threshold in this region.

Discussion

The exact histological basis for the three-layered appearance of arterial walls with the use of intravascular ultrasound imaging is controversial. Several groups have demonstrated variability in the three-layered ultrasound appearance while studying different arterial types with variable amounts of disease at frequencies of 20 MHz and lower.[12–15] The root of this controversy stems both from the use of different imaging systems (with different resolving power and dynamic range) and the variability between arterial types and extent of disease.

In peripheral vessels, the composition of the medial layer varies according to the location. Muscular arteries contain a media composed largely of smooth muscle cells with little collagen and elastin. This layer is poorly echo-reflective and forms a large acoustic mismatch between the surrounding layers, which results in a three-layered appearance on the ultrasound image.[16–18] Elastic vessels have a media that contains a large proportion of elastin and collagen, both of which are strongly echo-reflective. Thus, the acoustic impedance difference between layers is much less than in muscular vessels, and hence, distinct layering is not as clearly appreciated on the image. Because peripheral muscular arteries have a greater total thickness than coronary arteries, three layers can be observed by ultrasound even in nondiseased peripheral vessels. Coronary arteries are of the muscular type, with the exception of the most proximal portion of the left main artery, which is transitional in composition.

Natural history studies of the coronary vasculature have demonstrated that the degree of intimal thickening is age- and sex-related.[19–21] In age-matched subgroups, men in general have a thicker intimal layer than women.

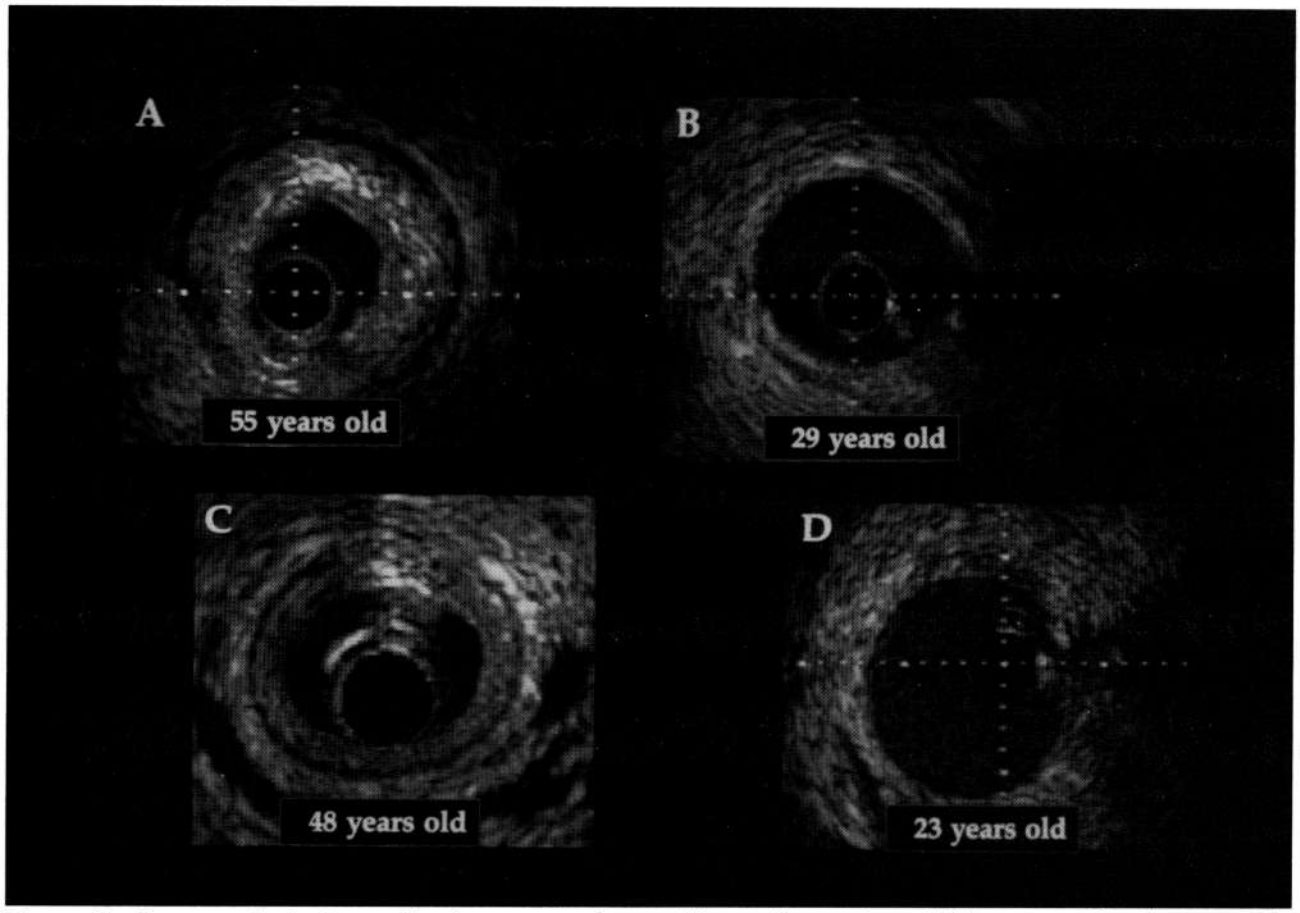

FIGURE 2. *Representative in vitro and in vivo coronary ultrasound images from patients of different ages. Clear layering on the ultrasound images is seen in the older group* (panels A *and* C), *but little evidence of layering is apparent in the younger hearts* (panels B *and* D).

The intimal layer thickens through adolescence and near the age of 30 years exceeds the dimensions of the underlying medial layer.[22] These same pathological studies show that the medial thickness does not change significantly in adolescents or young and mature adults. Thus, in young vessels and vessels with mild to moderate intimal thickness, the medial layer remains nearly constant and the overall thickness of the vessel is primarily a function of intimal growth. In advanced disease, the medial layer thins because of plaque encroachment and remodeling.

In our study, the histology and images from the 72 coronary cross sections showed similar findings to those pathological studies in adolescents and adults with mild disease. For the two groups (three-layered and nonlayered) the average medial thickness was nearly the same, yet the intimal thickness was significantly different. In addition, the individuals in the nonlayered group were younger than those in the three-layered group, which is consistent with the age-related changes observed in the prior postmortem studies. Thus, we would expect a significant proportion of adult patients with coronary disease presenting for cardiac catheterization to show a three-layered ultrasound appearance even in segments that may appear angiographically normal.

This study demonstrates that in young, nondiseased coronary vessels, both in vitro and in vivo imaging show lack of a three-layered appearance. In mildly diseased, older vessels, three layers are not observed until the intimal layer thickens sufficiently so that the overall thickness of the vessel wall becomes resolvable at 30 MHz. For the ultrasound system used in this study, this threshold was approximately 178 μm. Thus, the presence of three layers in coronary vessels, unlike the thicker muscular peripheral vessels, indicates the presence of disease.

The results of this study are dependent on the specific ultrasound system used. The 178-μm threshold will vary for different imaging systems because of different center frequencies and dynamic range. A lower frequency, such as 20 MHz, may require a greater intimal thickness before layering on the image becomes apparent. On the other hand, if a system with greater dynamic range were available, thinner intimal layers could probably be detected. In fact, the results of this study could also be interpreted as showing that early intimal thickening is acoustically more similar to media than later thickening; therefore, it may be the composition and not the thickness of the layer that determines its appearance. Visual inspection of the intimal tissue in our series does not support this interpretation, but further analysis of this issue with different ultrasound systems is clearly warranted. Preliminary clinical studies from other centers that used the same imaging system have confirmed the absence of layering in young transplant hearts.[23,24]

Although early, angiographically silent disease may be important in certain populations such as heart transplant recipients[25] and studies of plaque progression, this feature may not be of general clinical relevance. However, advancing atherosclerotic coronary disease leads to a progressive thinning of the media that may be very important to resolve. Gussenhoven et al[26] have reported that ultrasound is sensitive for detecting media that thins during moderate atherosclerotic disease. The ability to resolve medial borders is the first step in accurate sizing of lesions and may be useful in guiding therapeutic modalities such as atherectomy and laser to avoid penetration of this normal layer. In addition to system resolution being an important factor in recognizing medial borders, poor dynamic range can also lead to overestimation or underestimation of vessel layers and overall thickness.[27] Optimizing the dynamic range and the transducer frequency for the particular intravascular application is the first step toward meaningful image interpretation.

Conclusions

Intravascular imaging of coronary arteries with a frequency of 30 MHz demonstrates that the three-layered appearance corresponds to the presence of mild to moderate intimal thickening. Normal, nondiseased coronary arteries produce an ultrasound image with a homogeneous wall appearance without evident layering.

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